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MATERIALS AND METHODS

Statistical Design.—The test was designed to give results with as much significance as possible from a relatively small number of animals. The principle of "paired selection" was therefore applied. All animals were characterized as to breed type, sex, age, and weight, and were paired as closely as possible on this basis. Then one of each pair was assigned to the experimental (infested) group, while its mate was assigned to the controls (uninfested). All sheep were to be maintained under like conditions, leaving the single variable of keds to affect the results. In selecting sheep for sacrifice, complete pairs would make up the samples. Sampling was planned before infestation to detect any pre-existing skin defects in the flock, and was repeated at seven six-week intervals thereafter to clarify the time-related stages of progression and regression. The number of skins per sample varied from four to 12 pairs. Counting of both keds and cockle was to be as accurate as practical limitations permitted.

Test Sheep.—The test flock in New Mexico consisted of 150 Rambouillet crossbred sheep that had been born and raised under strictly controlled conditions to assure freedom from any known external parasite. Most of them (64 pairs) showed predominantly Rambouillet characteristics; others resembled the Cheviot (six pairs) or Hampshire (five pairs) features. At the start of the test, ages ranged from 0.5 to 6.5 years, but about 90 percent of the sheep were less than three years old (mostly 1.5 years). There were slightly more ewes (females) than wethers (castrated males). Each animal was identified by ear tags showing age, sex, and accession number. A plastic neck tag was added to show pairing for this test.

Sheep Maintenance.—Immediately after assignment the control sheep were confined in three adjacent pens of equal size. Those assigned to the infested group were placed in a similar set of pens some distance away. Infestation was performed only in these latter pens. Strict precautions were observed to prevent introducing any keds into the control pens. All pens were supplied with equal amounts of water and feed. The basic feed was a choice quality alfalfa hay. This was supplemented with pelleted feed (70 percent alfalfa, 20 percent milo maize, 10 percent molasses) as necessary to maintain good condition and gradual weight gain.

Ked Infestation.—Considerable difficulty was experienced in locating sources of keds during the warm fall months in New Mexico. Repeated searching at higher elevations finally yielded enough infested donor sheep for the purpose. A total of 40 adult keds was carefully placed close to the skin on each of the infested sheep, half of them on each side, during four infesting episodes between September 15 and November 2. At first they were placed at four locations: left and right shoulder; left and right rump. When later inspection revealed rapid migration to the forequarters, only the shoulder locations were used from then on. Suitable holding tables were used to restrain the sheep during the transfer operations.

Ked Counts.—A few days before slaughter, keds were counted on the infested sheep selected for sampling. This was done by restraining the animal, methodically parting the wool at closely spaced intervals, picking off all the adult keds, and recording their number. This was repeated over the entire body until no more keds could be found. Numbers of puparia were not recorded, since it was considered that only the mature insect could feed and thus cause cockle.

Regression of cockle was examined by manipulating exposure to keds in three equally-sized groups of sheep following the spring peak. In one group the keds remained unaltered to allow natural regression to occur. In the second group, keds were removed by shearing to simulate normal practice. In the third group, keds were killed by dipping the sheep in a vat containing a suspension of Cubé Powder (rotenone 5 percent) at the rate of one pound per 100 gallons of water. Thus the effect of ked removal was tested with and without the presence of long wool to determine whether or not the wool alone had any effect.

Pelt Recovery.—After slaughter at a local abattoir, identity of each pelt was assured by retaining the ear tag. The skins were spread out to cool, and were then well salted by applying about five pounds of clean, fine salt to the flesh side of each pelt, rubbing it into all areas by hand. Each pelt was then folded in half, rolled, and packaged in a plastic bag. The packages were shipped in fiber drums from Albuquerque to Philadelphia for evaluation, where they arrived in good condition after two to four weeks.

Skin Processing.—Since the primary objective was to evaluate unmodified cockle in pickled skins rather than to make the best leather, the usual reliming step was omitted and bating was minimal. Skins were painted with lime-sulfide paste and held overnight. After the wool was pulled, the skins were washed, delimed and bated for one hour at 85°F., washed again, and pickled normally. They were held in pickle overnight and then drained and lightly fleshed for counting. Further details were given previously (7).

The only exception to use of this standard method was in the case of Sample #4. For this sample we tried a modification suggested by a cooperating tanner. Dewooled skins were relimed for one day, washed with warm water, delimed and bated for two hours at 95°F., again washed with warm water, and pickled. This caused a significant reduction in cockle counts, as suggested many years ago (2, 14), and therefore invalidates use of these results for comparative purposes. However, it has led to additional studies on processing effects which will be the subject of a later report.

Cockle Counts.—While cockle is usually evident on the surface of pickled skins, the nodules are better visualized by means of strong transmitted light, as described previously (7). The skins were first marked off in a standard grid pattern, and numbers of cockle were recorded for each block of the grid.

RESULTS AND DISCUSSION

In the previous cockle survey (7), commercial salted woolskins were obtained at monthly intervals. The procedure was to cut the skins in half and process the left sides to the pickled stage. After evaluation of these, some of the matching right sides from each lot were selected for study before processing. Wool was clipped from the selected right sides and carefully searched. This approach re-

TABLE I

COMPARATIVE NUMBERS OF COCKLE AND ADULT KEDS ON SELECTED RIGHT SIDES OF COMMERCIAL SKINS

Cockle Grade	No. of Sides	Average Counts	
		Cockle	Keds
Light	4	21	3
Medium	6	850	105
Heavy	3	1,543	131

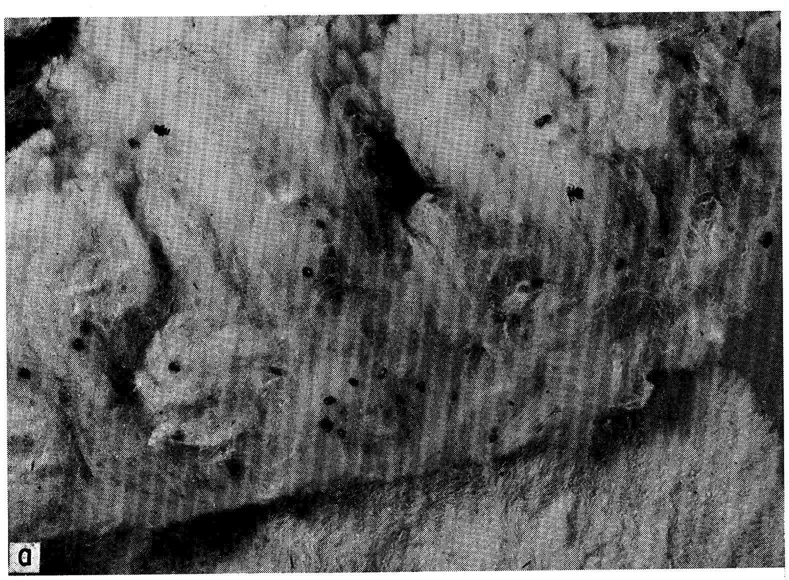

FIGURE 1a.—Keds and puparia in wool being clipped from shoulder area of salted woolskin with heavy cockle.

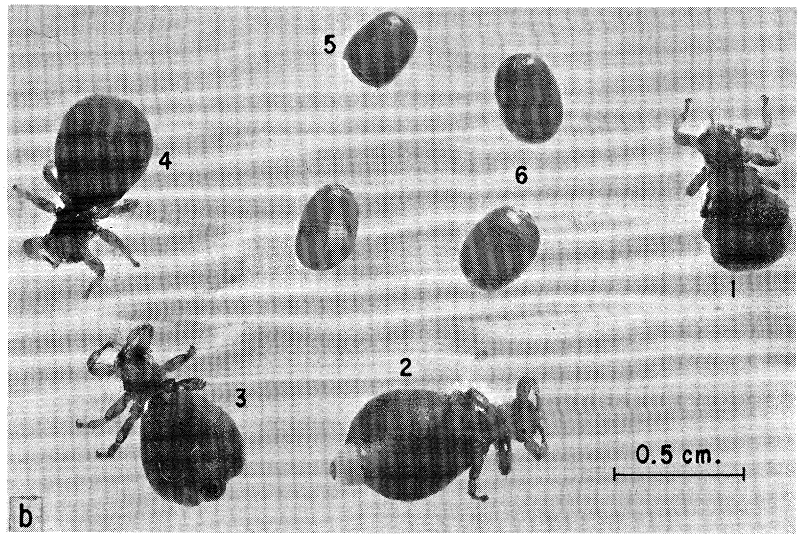

FIGURE 1b.—Freshly preserved keds: (1) probable male, others female; (2) female delivering single pupa; dorsal views (1) and (4), ventral (2) and (3); (5) empty puparium; (6) puparia containing developing pupae.

vealed the presence of varying populations of keds and suggested an apparent causal relationship with cockle. These observations are summarized in Table I. Average ked counts are shown along with cockle counts for the same sides, which are arranged according to an arbitrary grading system. The corresponding trends of increasing numbers of keds with severity of cockle is apparent and is further confirmed by results of the current test. An example of keds exposed during clipping is pictured in Figure 1a. Several preserved adults and puparia are shown with better detail in Figure 1b.

TABLE II

INDIVIDUAL COUNTS OF PAIRED SKINS IN SAMPLE #6

Pair No.	Infested Skins		Control Skins	
	Cockle	Keds	Cockle	Keds
42	1521	192	0	0
46	4045	275	169	0
47	842	700	215	0
52	1251	1600	0	0
53	1314	204	6	0
Average	1795	594	78	0
Median	1314	275	6	0

Seasonal Cycle of Cockle.—Considerable variation was encountered between individual skin counts within a sample, as is typical of most biological phenomena. This is illustrated in Table II, which shows actual cockle and ked counts from a representative sample. Obviously the average (mean) value is greatly influenced by a few extreme counts. The median value, or mid-point when counts are arranged in order, is more useful for showing trends because it is less

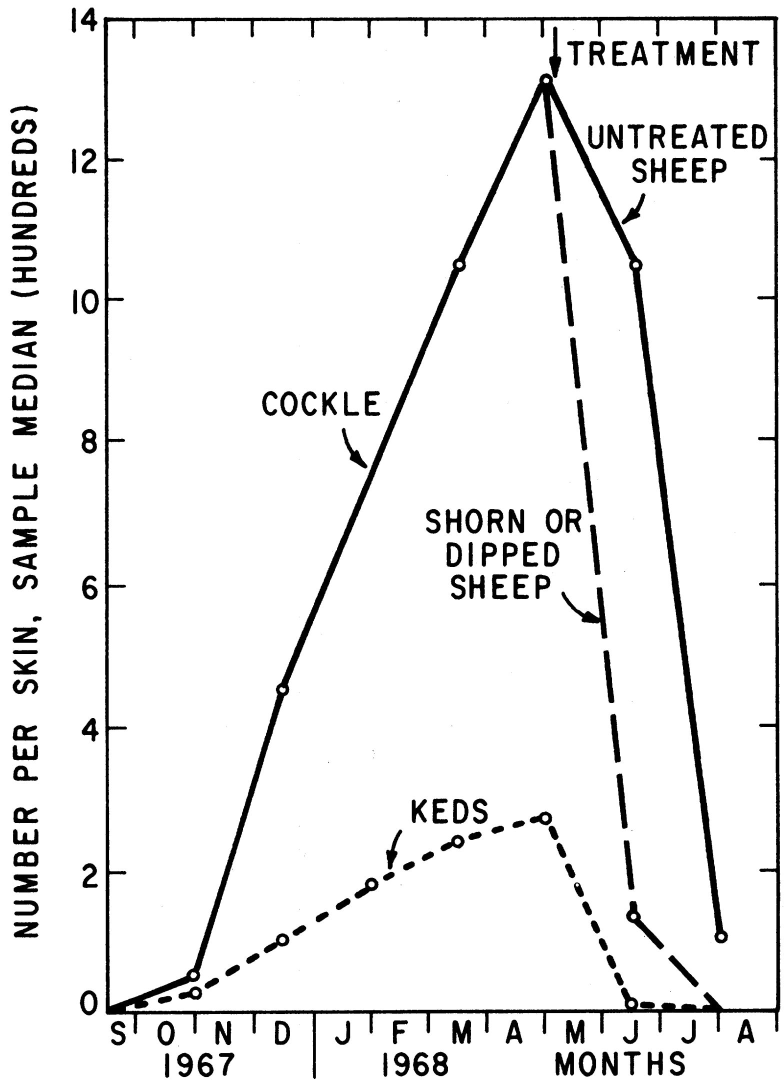

FIGURE 2.—Complete seasonal cycle of cockle and keds in sheep infested during September and October, showing spring peaks and faster regression from shearing or dipping.

TABLE III

SAMPLING SCHEDULE AND SUMMARY OF COCKLE AND KED COUNTS DURING CYCLE

Sample*	Time (Months)	Slaughter Date	No. of Skins		No. of Cockle per Skin			No. of Keds per Skin		
			Total†	Cockle	Median	High	Low	Median	High	Low
1-C	0	9/ 8/67	10	2	0	43	0	0		
2-I			4	4	53	205	19	31	38	22
2-C	1.5	11/ 2/67	4	1	0	15	0	0		
3-I			12	12	457	1114	117	107	174	59
3-C	3.0	12/18/67	12	2	0	15	0	0		
4-I‡			12	12	274	639	65	182	325	85
4-C‡	4.5	1/30/68	12	2	0	91	0	0		
5-I			11	11	1047	4670	278	245	535	61
5-C	6.0	3/12/68	10	5	5	1415	0	0		
6-I			5	5	1314	4045	842	275	1600	192
6-C	7.5	4/30/68	5	3	6	215	0	0		
7-I			3	3	1047	1207	622	7	17	7
7-C	9.0	6/19/68	3	1	0	50	0	0		
8-I			4	4	108	237	89	0		
8-C	10.5	7/31/68	4	3	10	45	0	0		
			Induced Regression — keds removed by shearing or dipping							
7-I-S			3	3	163	235	75	0		
7-C-S	9.0	6/19/68	3	1	0	21	0	0		
7-I-D			3	3	129	143	102	0		
7-C-D			3	2	12	20	0	0		
8-I-S			4	2	17	198	0	0		
8-C-S	10.5	7/31/68	4	2	3	139	0	0		
8-I-D			4	1	0	56	0	0		
8-C-D			4	2	31	160	0	0		

*C — control (uninfested); I — infested with 40 keds each from 9/15 to 11/2/67; S — shorn on 5/6/68; D — dipped in rotenone on 5/14/68.
†In Sample #5 the inequality is due to the unexplained death of a control sheep. Five pairs of skins from Sample #6 were lost in a processing accident.
‡Modified processing, used only on this sample, significantly reduced cockle counts and therefore invalidates comparative use of results (see section on skin processing).

influenced by extremes; therefore it will be used in most cases to interpret the results.

Table III summarizes data for the complete seasonal cycle, including number of skins, time schedule for each sample, incidence of cockle, and numbers of cockle and keds in terms of median value as well as range. While the time intervals were kept very constant, it was decided to decrease the size of some samples to make more animals available for the regression phase of the test. Partly because of its small size, statistical analysis of Sample #2 failed to show a highly significant difference (95 percent level of confidence) between cockle counts of control and infested skins. In Samples #3 to #7 inclusive, significance at this level was readily shown, which is felt to be sufficient evidence to prove the cockle-ked hypothesis, especially since every infested sheep developed cockle.

The cyclic severity of cockle proved to be a function of time of exposure to keds. Figure 2, where median values for cockle are plotted as the solid line, shows this in graphic form. It is clear that severity increased rapidly after the November sample and reached its peak with the April sample. From this point it decreased rapidly and had about disappeared by midsummer. This corresponds to the similar trend in numbers of keds, plotted as the dotted line.

Natural vs. Induced Regression.—From Figure 2 it can also be seen that removal of keds, by shearing and by dipping, appreciably hastened the rate of cockle regression as compared with the untreated sheep, but it still took about two months for the cockle to disappear. This is appreciably longer than estimates by Seymour-Jones (2). Again the sample sizes were small, and differences are not highly significant when analyzed as three separate treatments. It was considered reasonable to combine values for shorn and dipped samples as shown by the broken line in the graph; the differences then became more significant. Table III indicates that regression of cockle was slightly more rapid after dipping than after shearing, which probably reflects the fact that small numbers of keds managed to survive in the wool stubble for about a week after shearing. The presence of the wool had no apparent effect on the rate of regression.

Host-Parasite Interaction.—Extreme variations among numbers of both cockle and keds within samples, as illustrated in Table II, made it impossible to show statistically that the two were directly correlated on an individual animal basis, although sample trends were obvious (Figure 2). Table IV shows the extent of this variability when calculated as standard deviations. In order to compensate for the varying size of the skins, the cockle and ked counts were calculated on the basis of skin weight. It was considered that these values would approximate the concentration per unit of skin area. In spite of this attempt at refinement, the variability remained extremely large.

It is apparent that complex interactions take place between the parasitic keds and their hosts, and more information is needed to clarify this relationship. For

TABLE IV

VARIABILITY IN NUMBERS OF COCKLE AND KEDS WITHIN SAMPLES

Sample*	No. of Skins	No. of Cockle per kg.**		No. of Keds per kg.**	
		Average	S. D.	Average	S. D.
2	4	25	22	10	3.6
3	12	258	181	56	24
4	12	131	86	116	59
5	11	603	515	119	77
6	5	849	693	291	324
7	3	413	146	4.4	2.6
8	4	57	31	0	

*Skins from infested sheep during natural cycle.
**Based on skin weight immediately after dewooling; S. D. — standard deviation.

one thing, ked populations appear to be very dynamic and unpredictable on any given animal. A single count just before slaughter would not reflect the changes that had occurred previously. Also there is growing evidence that many sheep develop a natural resistance to keds after a period of exposure (15). This is reported (16) to cause constriction of the blood capillaries just below the skin surface, reducing the blood supply available to the keds and thus reducing their numbers. Further testing is required to establish the over-all mechanism by which keds cause cockle and to resolve the roles of the various physiological and pathological factors which affect the numbers of keds and cockle nodules.

Distribution of Cockle.—The characteristic location and extent of cockle areas on a sheepskin at different degrees of severity are essential points in defining the defect. It is commonly believed that cockle starts on the neck and shoulders and spreads from there towards the rear and downwards. The controlled conditions of this test provided the data for accurate documentation of this sequence during its entire cycle. Figure 3 graphically shows four stages in the progressive development of cockle to its spring peak of severity, while Figure 4 shows two stages in its summer regression, under natural conditions as well as when it is hastened by shearing or dipping. The dots represent average cockle counts in a given block in the grid pattern for all skins of that sample; small dots stand for one cockle spot, while large dots are equivalent to five. Thus each figure is a composite diagram representing a particular sample of infested skins.

It can be seen (Figure 3a) that development of cockle began mainly in the shoulder and chest region as expected, since keds seem to prefer this area for some unknown reason (17, 18). As ked numbers increased, the cockle (Figure 3b) built up rapidly in the shoulders and neck and began spreading towards the rear. At a later stage (Figure 3c) the distribution became more uniform and the concentration increased everywhere. At its peak of severity (Figure 3d) the cockle

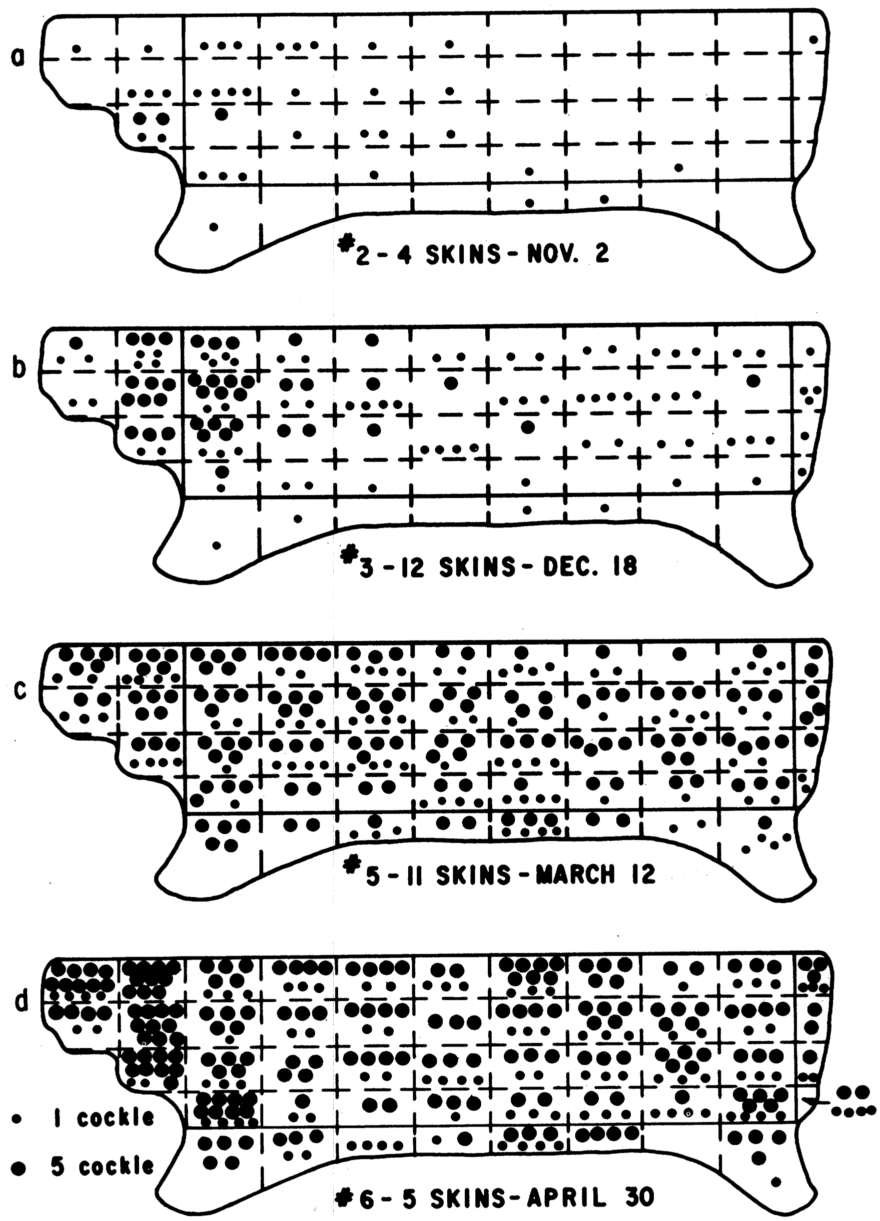

FIGURE 3.—Composite diagrams showing distribution of cockle on left sides of infested sheep at four stages of development. Dots represent average counts within each block for each sample: (a) early preference for shoulder and chest; (b) continued concentration in shoulder with spreading to rear; (c) widespread involvement; (d) final increase in density at spring peak.

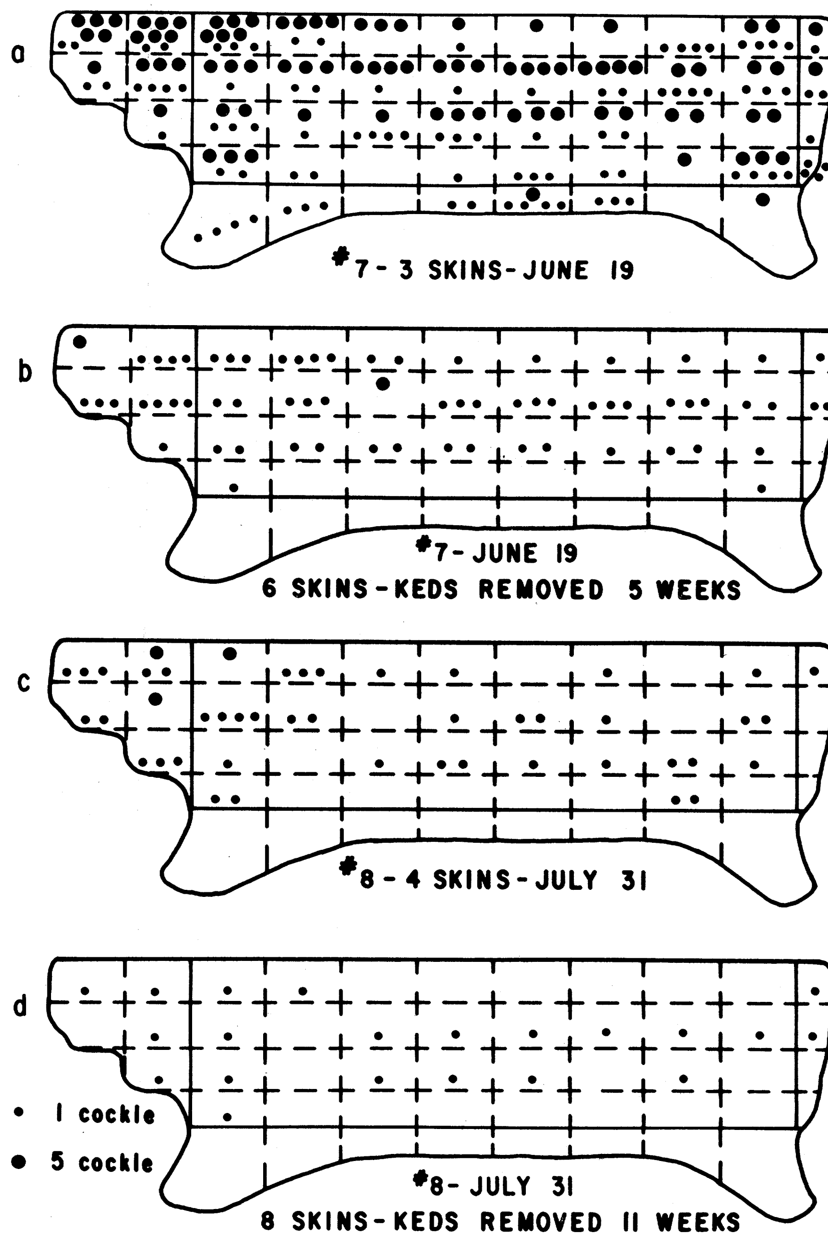

FIGURE 4.—Composite diagrams of cockle distribution during summer regression: (a) rapid generalized decrease from Figure 3d as keds succumbed to heat; (b) more rapid decrease from abrupt removal of keds by shearing or dipping; (c) continued decrease from (a) but still some preference for shoulder; (d) essential disappearance of cockle 2.5 months after treatment.

was widely distributed and the nodules were crowded closely together in many areas. During natural regression (Figure 4a), the concentration decreased rapidly in all areas as ked populations diminished. Where sheep had been shorn or dipped, the rate of reduction was even more rapid (Figure 4b). The final stage of natural regression (Figure 4c) still showed a lingering preference for neck and shoulders, while the induced regression (Figure 4d) resulted in a more uniform and abrupt finish.

Symmetry of Cockle.—Space limitations prevent inclusion of diagrams of the right sides matching those shown in Figures 3 and 4, which would have given a detailed comparison for assessing symmetry. In general there was usually a close similarity in appearance between left and right sides of skins with cockle, especially in the later stages of development. Table V illustrates this similarity in numerical terms, comparing front and rear halves of each side as well as the side totals, at various stages of development and regression. For some unknown reason the left sides showed a slight but consistent trend to higher numbers than the right sides. This difference was statistically significant in Samples #5 and #6. Also the front quadrants always had higher counts than those from the rear, but this is to be expected, since the former region includes the neck portion and has a larger area. A similar pattern of symmetry was reported in the previous study of commercial skins (7, Table IV).

TABLE V

SYMMETRY OF COCKLE AT VARIOUS STAGES OF CYCLE

Sample	Months	Average Cockle Counts by Quadrants					
		Left Side			Right Side		
		Front	Rear	Total	Front	Rear	Total
			Early Development				
2	1.5	42	4	46	28	9	37
3	3.0	229	48	277	234	38	272
			Late Development				
5	6.0	488	308	796	394	254	648
6	7.5	601	446	1047	402	345	747
			Natural Regression (undisturbed)				
7	9.0	311	211	522	271	166	437
8	10.5	44	15	59	54	23	77
			Induced Regression (shorn or dipped)				
7	9.0	47	24	71	44	27	71
8	10.5	13	7	20	10	6	16

Appearance of Cockle.—In describing distribution and symmetry of cockle, numbers alone tell only part of the story. During the later stages cockle nodules form distinctive distribution patterns characterized by rows running perpendicular

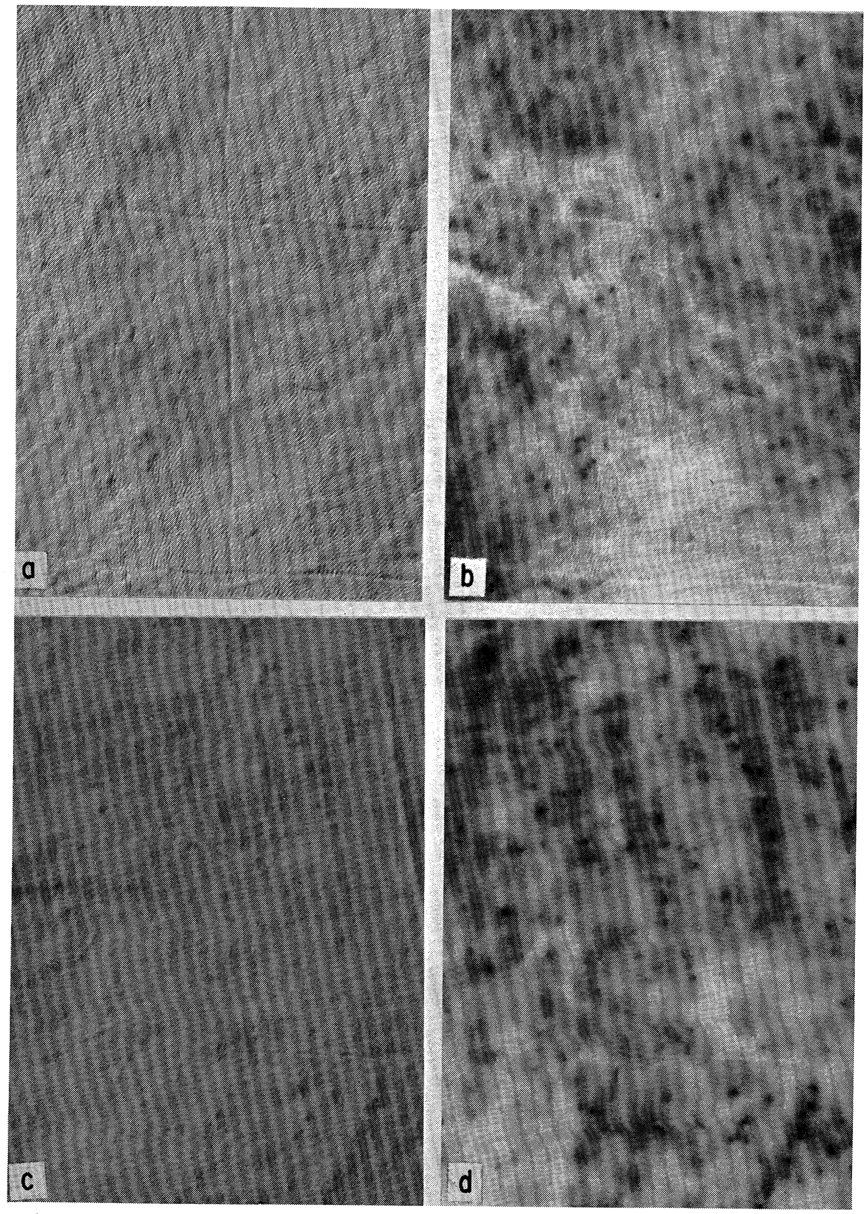

FIGURE 5.—Appearance of cockle in pickled skins from infested sheep. Upper figures: skin from Sample #2 with patch of light cockle in shoulder; lower figures: skin from Sample #5 with heavy cockle just behind shoulder. Left views by reflected light; right views by transmitted light to illustrate actual appearance during counting.

to the backbone, particularly in the mid-section. This was well illustrated in the previous publication (7, Figure 3). During its earlier stages, cockle tends to form irregular patches, mostly in the neck and shoulders, and scattered spots elsewhere. Figure 5 illustrates known early and late stages of cockle in the test skins. As seen by reflected light (Figure 5a), a shoulder patch of early cockle is relatively inconspicuous, since many of the nodules are not well developed. By transmitted light (Figure 5b), many additional spots become visible because of their density to light. At later stages (Figure 5c), most of the nodules are much larger and darker in color. Their extreme variation in size can best be appreciated by means of transmitted light (Figure 5d).

Animal Factors.—A question of interest is whether the severity of cockle was influenced by such factors as: breed, sex, age, nutrition, or weight of the sheep. The test sheep were so closely similar in most of these respects that only the sex factor could be evaluated. In three of the seven samples of infested sheep there was significantly more cockle in skins from males; in two others the skins from females were favored. The remaining two samples were essentially alike. On the basis of these limited numbers, there was no obvious difference between the sexes.

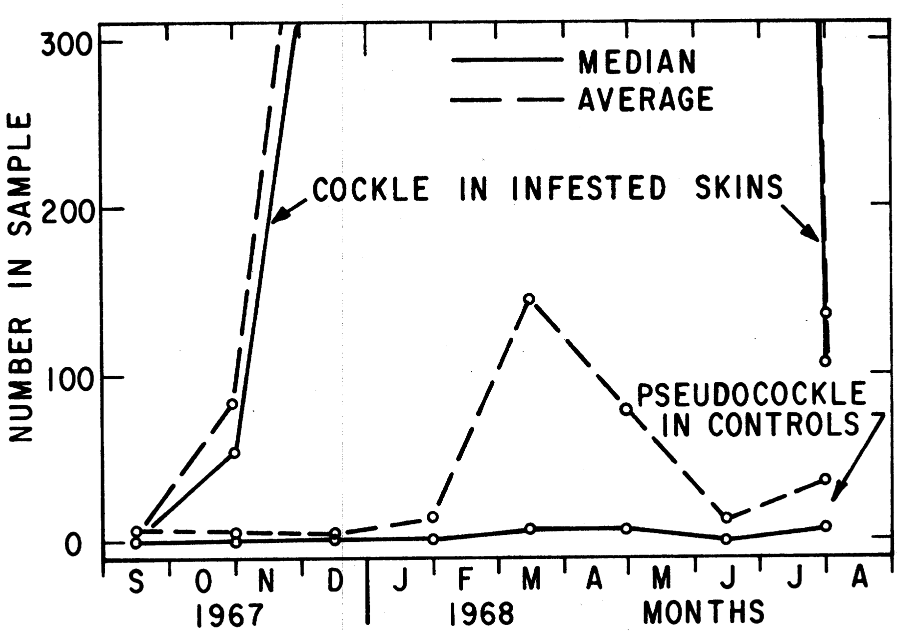

FIGURE 6.—Comparison of pseudocockle (a condition resembling cockle but not caused by keds) in control skins, with true cockle in infested skins. Median curve (bottom) shows no significance; peak in average curve due to single abnormal skin. Curve for cockle reproduced in part from Figure 2, with vertical scale doubled.

Pseudocockle in Controls.—A point of some concern has been the persistent occurrence of "apparent" cockle, usually in low numbers, in the uninfested skins. Several lines of evidence strongly indicate that this condition is not the same as the "true" cockle found in the infested skins, hence the term "pseudo." (*a*) It was present in two skins of the initial sample (Table III, Sample 1-C) before infestation, thus showing it had no relation to keds or other known ectoparasites. (*b*) It is apparent from Figure 6 that it never reached significant levels in terms of median numbers (solid line at bottom). Even in terms of average numbers (Figure 6, broken line), the shape of the curve bears no resemblance to that for true cockle, confirming that keds were not involved. The peak of the curve is due to a single "wild" or highly abnormal skin in Sample #5. Except for this skin, the highest count was 215. (*c*) The nodules of pseudocockle were usually white rather than brownish, and their distribution patterns were not typical of the true cockle induced by keds. Histological investigation at a later date should help to resolve the nature of pseudocockle. It is also planned to convert many of the test skins into a type of leather suitable for critical evaluation of cockle, which should supply additional helpful evidence.

CONCLUSIONS

The test described was successful in reproducing a complete seasonal cycle of the cockle defect. Nodules, or papules, within the skin were shown to be concentrated over neck and shoulder regions during fall and early winter, subsequently advancing over the entire body surface; the condition attained its peak of severity during the spring months, and disappeared naturally with the advent of summer. Shearing or dipping accelerated the rate of its disappearance. The nature of this pathological condition was completely typical of that found in commercial skins.

Differences in severity of cockle between infested and control skins reached high levels of significance in every sample after the first infested one, and without exception every infested animal developed cockle. Coupled with virtual absence of cockle (as indicated by median numbers) in the uninfested controls, this seems to establish conclusive proof of the cockle-ked hypothesis.

It should be emphasized that the climatic environment of this test in New Mexico is different from that of the majority of important sheep-raising states, and might well have had a significant influence on some of the results obtained.

This test raises some interesting speculations about the host-parasite interactions. The number of cockle nodules could not often be directly correlated with the ked population at a given time on a specific animal. Reasons for this variable tissue response between different animals, and the mechanism of the formation of cockle nodules, are challenging questions in pathology. The whole problem deserves further study.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. W. C. Stewart, of Temple University, for statistical assistance in planning the test and interpreting the results; to M. C. Audsley and staff, of EURDD, for preparing the figures used in the manuscript; and to the A. C. Lawrence Leather Co. for advice on processing methods. Mr. Yule B. Reese, of Wool Warehouse Co., Albuquerque, kindly assisted in the selection and pairing of sheep.

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NOTE: Following the completion of this manuscript it was learned that a similar conclusion had been reached independently at the Centre Technique du Cuir in Lyon, France. Their results, culminating five years of research, traced the origin of cockle to *Melophagus ovinus* and showed the beneficial effect of insecticidal treatment (M. Laidet. *Technicuir,* 3, 9–20, April, 1969).